Inhibition of *Clostridium botulinum* by Aliphatic Amines and Long Chain Aliphatic Aminodiamides

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ABSTRACT

N-substituted aminodiamides of the general structure, $RNHCO(CH_2)_x\text{-}CONH(CH_2)_yN(CH_3)_2$, and the corresponding N-sulfopropyl derivatives were studied for their inhibiting effect on growth of *Clostridium botulinum* in culture media. With R = $C_{16}H_{33}$, more activity was observed with x = 2, 3, or 4 and y = 2, 3, or 4 than with R = $C_{12}H_{25}$ or $C_{10}H_{21}$. There was little or no correlation of activity with the length of the x or y chains, but the addition of the sulfopropyl group resulted in decreased inhibitory activity. The C_3 to C_{18} saturated aliphatic amines used for synthesizing the aminodiamides were effective inhibitors of *C. botulinum*; tetradecyl-, pentadecyl-, and hexadecylamines were the most active with minimum inhibitory concentrations (MIC) of 0.8 μg/ml. The ratio of molecular weights of the diamides to the corresponding aliphatic amines indicated that most if not all of the inhibitory activity of the aminodiamides could be attributed to the amine moiety.

INTRODUCTION

The inhibition of various gram positive and gram negative bacteria by aliphatic fatty acids was reviewed by Neiman (1). Subsequent studies by Galbraith et al. (2) showed lauric acid (C_{12}) to be the most active saturated fatty acid against gram positive bacteria. Eisler and von Metz (3) also found *Pasteurella pestis* to be strongly inhibited by C_{12} and C_{14} saturated fatty acids. Borick et al. (4) indicated that saturated fatty acid salts of tetradecylamine were active against a variety of microorganisms, while Borick and Bratt (5) showed that higher amine salts of polycarboxylic acids were also capable of inhibiting many bacteria.

Sulfopropylated derivates of alkylsuccinamide were synthesized by Micich et al. (6); Micich and Linfield (7) synthesized sulfobetaine derivatives of N-alkylglutaramide and adipamide. These compounds are being investigated in our laboratories as potential soap-based general bactericidal agents, but we are also interested in possible uses of such compounds in foods. This report deals with the inhibitory properties of these compounds in culture media against *C. botulinum*.

TABLE I

Inhibition of *C. botulinum* by N-substituted aminodiamides

Index compound $RNHCO(CH_2)_xCONH(CH_2)_yN(CH_3)_2$			MIC[a] (μg/ml) where R =		
x	y		$C_{10}H_{21}$	$C_{12}H_{25}$	$C_{16}H_{33}$
2	2	$(CH_2)_3SO_3^-$[b]	12.5	>50[c]	12.5
2	3	↓	12.5	50	3.1
2	4		ND[d]	ND	3.1
3	2		>50	12.5	12.5
3	3		>50	>50	12.5
4	2		12.5	>50	0.8
4	3		50	>50	12.5
4	4		ND	ND	50
2	2		50	1.6	ND
2	3		ND	12.5	ND
2	4		ND	ND	1.6
3	2		12.5	1.6	1.6
3	3		>50	3.1	1.6
4	2		50	3.1	1.6
4	3		50	12.5	ND
4	4		ND	ND	1.6

[a]MIC = minimum inhibitory concentration.
[b]$(CH_2)_3SO_3^-$ on the dimethylamino group.
[c]The highest level tested was 50 μg/ml.
[d]ND = not determined.

EXPERIMENTAL PROCEDURES

The assay medium of Huhtanen (8) was used in 5 ml amounts in 15 x 125 mm test tubes incubated at 37 C in a National Appliance Co. controlled environment chamber with evacuation to 26 in. Hg (100 torr) followed by nitrogen replacement. The compounds were dissolved in alcohol, and a series of $\log_2$ dilutions was made in the assay tubes starting with 50 μg/ml.

The inoculum was one drop per tube of a 1:50 dilution, in assay medium, of a 24-h culture grown in the same medium. Inhibition was determined by visual inspection for turbidity, with verification, in the case of insoluble compounds, by plunging a hot loop in the medium and observing gas evolution.

RESULTS AND DISCUSSION

The index aminodiamide was $RNHCO(CH_2)_xCONH(CH_2)_yN(CH_3)_2$. A sulfopropyl group was added to the amino function in some cases. The effect of this grouping on the inhibition of *C. botulinum* is shown in Table 1. Generally, there was less inhibition with the sulfopropyl group. This was most pronounced where R was $C_{12}H_{25}$ but was also quite evident when R was $C_{16}H_{33}$.

The least active R group was the decyl ($C_{10}H_{21}$), especially when no sulfopropyl group was present. Dodecyl ($C_{12}H_{25}$) was more active, and hexadecyl ($C_{16}H_{33}$) was the most inhibitory without the sulfopropyl group. With the sulfopropyl group present, the dodecyl and decyl derivatives were about equal except when 2 or 4 (but not 3)-CH_2-groups separated the two amides; in these cases the decyl group appeared to give slightly greater inhibition.

The most active compounds, giving a minimum inhibitory concentration (MIC) of 0.8 μg/ml, were those with R = $C_{16}H_{33}$ containing no sulfopropyl group.

The length of the methylene chain separating the two amides or the dimethylamine from its neighboring amide did not influence the MIC.

The greater inhibition of the dodecyl and hexadecyl-derived diamides suggested a relationship to the previously observed inhibition of various bacteria by saturated fatty acids. Lactobacilli were shown by Hassinen et al. (9) to be most strongly inhibited by dodecanoic acid, while Eisler and von Metz (3) found dodecanoic, tetradecanoic, and hexadecanoic acids to be the most effective against *Pasteurella pestis*. Galbraith et al. (2) also showed dodecanoic acid to

be the most active against *C. butyricum* and *C. sporogenes*. We tested these fatty acids against *C. botulinum;* none was active at 50 μg/ml.

Because aliphatic amines are more soluble than fatty acids, we determined the relative effectiveness of a series of these amines for inhibiting *C. botulinum*. These included the C_{10}, C_{12}, and C_{16} aliphatic amines used in the preparation of the aminodiamides. The results are shown in Table 2. There was little or no inhibition up to and including the C_9. From C_{10} to C_{14} there was generally an increase in activity with increasing chain length. The amines with 14, 15, and 16 carbons showed the same MIC (0.8 μg/ml), while the octadecylamine (C_{18}) showed only slightly less inhibition (MIC = 1.6 μg/ml).

A comparison of the inhibition by the C_{16} aminodiamides and hexadecylamine (Tables I and II) indicated that the amine was about twice as active. Since the amine contributed approximately one-half the molecular weight of the diamide, it is possible that this is the source of the inhibition noted. Possibly the diamides could be hydrolyzed in the cells releasing the active amine. Approximately the same ratios of activities to molecular weights were evident also for the C_{10} and C_{12} compounds.

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